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(54) Title: CRYSTALLIZATION MEDIA			
(57) Abstract			
<p>In one aspect, the present invention provides crystallization solutions useful for crystallizing proteins and other molecules, especially macromolecules. In a presently preferred embodiment, the crystallization solutions of the invention are combined as four crystallization solution sets. Each of the four crystallization solution sets includes forty eight different crystallization solutions. Each individual crystallization solution includes a precipitant and a buffer, and optional includes at least one additive. In another aspect, the present invention provides kits including a plurality of crystallization solutions of the present invention and at least one crystallization plate that preferably includes a plurality of reservoirs. Preferably the crystallization solutions are disposed within the reservoirs of the crystallization plates.</p>			
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CRYSTALLIZATION MEDIA

Field of the Invention

The present invention relates to solutions that are useful in the crystallization of molecules, especially macromolecules such as proteins.

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Background of the Invention

Macromolecular X-ray crystallography is an essential tool in modern drug discovery and molecular biology. Using X-ray crystallographic techniques, the three-dimensional structures of biological macromolecules, such as proteins, nucleic acids, and their various complexes, can be determined at practically atomic-level resolution
10 from X-ray diffraction data.

One of the first and most important steps in the X-ray crystal structure determination of a target macromolecule is to grow large, well-diffracting crystals of the macromolecule. As the techniques for collecting and analyzing X-ray diffraction data have become more rapid and automated, crystal growth has become a rate-limiting step in the structure determination process.
15

Vapor diffusion is the most widely used technique for crystallization in modern macromolecular X-ray crystallography. In this technique, a small volume of the macromolecule sample is mixed with an approximately equal volume of a crystallization solution. The resulting drop of liquid (containing macromolecule and
20 dilute crystallization solution) is sealed in a chamber with a much larger reservoir volume of the crystallization solution. The drop is kept separate from the reservoir of crystallization solvent either by hanging the drop from a glass cover slip or by sitting the drop on a pedestal above the level of the solvent in the reservoir. Over time, the crystallization drop and the reservoir solutions equilibrate via vapor diffusion of

volatile chemical species. Supersaturating concentrations of the macromolecule are achieved, resulting in crystallization of the macromolecule sample in the drop.

The process of growing biological macromolecule crystals remains, however, a highly empirical process. Macromolecular crystallization is dependent on a host of experimental parameters, including; pH, temperature, the concentration of salts in the crystallization drop, the concentration of the macromolecule to be crystallized, and the concentration of the precipitating agent (of which there are hundreds). In particular, the choice of solute conditions in which to grow crystals continues to be a matter for empirical determination. Consequently, the ability to rapidly and easily generate many crystallization trials is important in determining the ideal conditions for crystallization. Thus, there is a need for sets of preformulated crystallization solutions that can be used to rapidly and easily generate many crystallization trials.

Summary of the Invention

In one aspect, the present invention provides solutions (hereinafter referred to as crystallization solutions) useful for crystallizing proteins and other molecules, especially macromolecules. In a presently preferred embodiment, the crystallization solutions of the invention are combined as four crystallization solution sets (identified herein as Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV). Each of the four crystallization solution sets includes forty eight different crystallization solutions. Each individual crystallization solution includes a precipitant and a buffer, and optionally includes at least one additive as set forth more fully herein. The compositions of the individual solutions that constitute Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV are set forth in Table I, Table II, Table III and Table IV, respectively. Thus, in one aspect, the present invention is directed to sets of crystallization solutions including the solutions of one or more of Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV.

In another embodiment, the present invention provides subsets of the crystallization solutions of Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV which are useful to determine crystallization conditions for a wide variety of molecules, especially biological macromolecules. While any subset of crystallization solutions of Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV can be utilized, presently preferred

crystallization solution subsets are: subset I, solution numbers 1, 9, 10 and 28 of Crystallization Solution Set I; subset II, solution numbers 1, 8, 14, 26, 30 and 34 of Crystallization Solution Set II; subset III, solution numbers 2, 11, 18, 25, 26, 28, 29, 31, 38, 41 and 46 of Crystallization Solution Set III; and subset IV, solution numbers 5 2, 3, 5, 6, 7, 8, 17, 22, 24, 32, 34, 37, 43 and 48 of Crystallization Solution Set IV. Thus, crystallization solution subsets preferably include at least one of subset I, subset II, subset III and subset IV. As described more fully herein, the foregoing, presently preferred subsets have been successfully used to crystallize several proteins, including proteins considered recalcitrant to crystallization.

- 10 In another aspect, the present invention provides kits including a plurality of crystallization solutions of the present invention and at least one crystallization plate that preferably includes a plurality of reservoirs. Preferably the crystallization solutions of the crystallization solution set(s) are disposed within the reservoirs of the crystallization plates. The presently preferred crystallization plates are disclosed in
15 copending US Patent Application Serial Number 09/150,629, incorporated herein by reference. In a presently preferred embodiment, the present invention provides kits including at least one crystallization plate and a set of crystallization solutions selected from the group of sets consisting of Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV.
20 In another presently preferred embodiment, the present invention provides kits including at least one crystallization plate and including a subset of crystallization solutions selected from the group of subsets consisting of: subset I, solution numbers 1, 9, 10 and 28 of Crystallization Solution Set I; subset II, solution numbers 1, 8, 14, 26, 30 and 34 of Crystallization Solution Set II; subset III, solution numbers 2, 11,
25 18, 25, 26, 28, 29, 31, 38, 41 and 46 of Crystallization Solution Set III; and subset IV, solution numbers 2, 3, 5, 6, 7, 8, 17, 22, 24, 32, 34, 37, 43 and 48 of Crystallization Solution Set IV.

- Thus, the present invention provides solutions and kits that permit a large number of crystallization conditions to be easily and simultaneously tested in order to
30 identify crystallization conditions under which a target molecule, especially a biological macromolecule such as a protein, can be crystallized. As discussed more fully herein, the crystallization solutions of the present invention have been successfully used to crystallize proteins regarded as recalcitrant to crystallization.

Brief Description of the Drawings

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the 5 accompanying drawings, wherein:

FIGURE 1 is a three dimensional view of a presently preferred crystallization plate useful for inclusion in a kit of the present invention.

FIGURE 2 is a view of the upper surface of a presently preferred crystallization plate useful for inclusion in a kit of the present invention.

10 Detailed Description of the Preferred Embodiment

In one aspect, the present invention provides crystallization solutions useful for crystallizing proteins and other molecules, especially macromolecules. In a presently preferred embodiment, the crystallization solutions of the invention are combined as four crystallization solution sets (identified herein as Crystallization 15 Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV). Each of the four crystallization solution sets includes forty eight different crystallization solutions. Each individual crystallization solution includes a precipitant, a buffer and optionally an additive. The compositions of the individual solutions that constitute Crystallization Solution Set I, Crystallization 20 Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV are set forth in Tables I, II, III and IV, respectively. The following abbreviations are used: CAPS - 3-(cyclohexylamino)-1-propanesulfonic acid; CHES - 2-(N-cyclohexylamino)ethanesulfonic acid; HEPES - N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid); MES - 2-(N-morpholino)ethanesulfonic acid; MME - 25 monomethyl ether; OAc - acetate; PEG - polyethylene glycol; Tris - tris(hydroxymethyl)aminomethane.

Table I
Compositions of the Crystallization Solutions of Crystallization Solution Set I

Solution No.	precipitant	buffer (0.1 M)	additive(s)
1	40% (v/v) 2-methyl-2,4-pentanediol	phosphate-citrate pH 4.2	none
2	40% (v/v) ethylene glycol	acetate pH 4.5	none
3	50% (v/v) PEG-200	citrate pH 5.5	none

Solution No.	precipitant	buffer (0.1 M)	additive(s)
4	40% (v/v) PEG-300	HEPES pH 7.5	0.2 M NaCl
5	40% (v/v) PEG-400	citrate pH 5.5	0.2 M MgCl ₂
6	40% (v/v) PEG-600	cacodylate pH 6.5	0.2 M Ca(OAc) ₂
7	40% (v/v) ethanol	Tris pH 8.5	0.05 M MgCl ₂
8	35% (v/v) 2-ethoxyethanol	cacodylate pH 6.5	none
9	35% (v/v) 2-propanol	phosphate-citrate pH 4.2	none
10	45% (v/v) glycerol	imidazole pH 8.0	none
11	35% (v/v) 2-methyl-2,4-pentanediol	Tris pH 8.5	0.2 M (NH ₄) ₂ SO ₄
12	50% (v/v) ethylene glycol	acetate pH 4.5	5% (w/v) PEG-1000
13	30% (v/v) PEG-200	MES pH 6.0	5% (w/v) PEG-3000
14	20% (v/v) PEG-300	phosphate-citrate pH 4.2	0.2 M (NH ₄) ₂ SO ₄ , 10% (v/v) glycerol
15	50% (v/v) PEG-400	CHES pH 9.5	0.2 M NaCl
16	30% (v/v) PEG-600	MES pH 6.0	5% (w/v) PEG-1000, 10% (v/v) glycerol
17	40% (v/v) 1, 2-propanediol	HEPES pH 7.5	none
18	35% (v/v) 2-ethoxyethanol	imidazole pH 8.0	0.05 M Ca(OAc) ₂
19	35% (v/v) 2-propanol	Tris pH 8.5	none
20	30% (v/v) 1,2-propanediol	citrate pH 5.5	20% (v/v) 2-methyl-2,4-pentanediol
21	40% (v/v) 1,2-propanediol	acetate pH 4.5	0.05 M Ca(OAc) ₂
22	40% (v/v) ethylene glycol	Na/K phosphate pH 6.2	none
23	40% (v/v) 2-methyl-2,4-pentanediol	Tris pH 7.0	0.2 M (NH ₄) ₂ SO ₄
24	40% (v/v) PEG-400	Na/K phosphate pH 6.2	0.2 M NaCl
25	30% (v/v) PEG-200	Tris pH 8.5	0.2 M (NH ₄) ₂ HPO ₄

Solution No.	precipitant	buffer (0.1 M)	additive(s)
26	40% (v/v) PEG-300	CHES pH 9.5	0.2 M NaCl
27	30% (v/v) PEG-400	CAPS pH 10.5	0.5 M $(\text{NH}_4)_2\text{SO}_4$, 10% (v/v) glycerol
28	30% (v/v) PEG-600	HEPES pH 7.5	0.05 M Li_2SO_4 , 10% (v/v) glycerol
29	40% (v/v) PEG-300	CHES pH 9.5	0.2 M sodium citrate
30	35% (v/v) 2-ethoxyethanol	citrate pH 5.5	none
31	35% (v/v) 2-propanol	citrate pH 5.5	5% (w/v) PEG-1000
32	40% (v/v) 1,2-propanediol	CHES pH 9.5	0.2 M sodium citrate
33	25% (v/v) 1,2-propanediol	imidazole pH 8.0	0.2 M $\text{Zn}(\text{OAc})_2$, 10% (v/v) glycerol
34	40% (v/v) 2-methyl-2,4-pentanediol	imidazole pH 8.0	0.2 M MgCl_2
35	40% (v/v) ethylene glycol	HEPES pH 7.5	5% (w/v) PEG-3000
36	50% (v/v) PEG-200	Tris pH 7.0	0.05 M Li_2SO_4
37	40% (v/v) PEG-300	cacodylate pH 6.5	0.2 M $\text{Ca}(\text{OAc})_2$
38	40% (v/v) PEG-400	Tris pH 8.5	0.2 M Li_2SO_4
39	40% (v/v) PEG-600	phosphate-citrate pH 4.2	none
40	40% (v/v) ethanol	phosphate-citrate pH 4.2	5% (w/v) PEG-1000
41	25% (v/v) 1,2-propanediol	phosphate-citrate pH 4.2	5% (w/v) PEG-3000, 10% (v/v) glycerol
42	40% (v/v) ethylene glycol	Tris pH 7.0	none
43	50% (v/v) ethylene glycol	Tris pH 8.5	0.2 M MgCl_2
44	50% (v/v) PEG-200	cacodylate pH 6.5	0.2 M $\text{Zn}(\text{OAc})_2$
45	20% (v/v) PEG-300	Tris pH 8.5	5% (w/v) PEG-8000, 10% (v/v) glycerol

Solution No.	precipitant	buffer (0.1 M)	additive(s)
46	40% (v/v) PEG-400	MES pH 6.0	5% (w/v) PEG-3000
47	50% (v/v) PEG-400	acetate pH 4.5	0.2 M Li ₂ SO ₄
48	40% (v/v) PEG-600	imidazole pH 8.0	0.2 M Zn(OAc) ₂

Table II
Compositions of the Crystallization Solutions of Crystallization Solution Set II

Solution No.	precipitant	buffer (0.1 M)	additive(s)
1	40% (v/v) 2-methyl-2,4-pentanediol	cacodylate pH 6.5	5% (w/v) PEG-8000
2	50% (v/v) PEG-200	CHES pH 9.5	none
3	40% (v/v) ethylene glycol	phosphate-citrate pH 4.2	0.2 M (NH ₄) ₂ SO ₄
4	40% (v/v) PEG-400	HEPES pH 7.5	0.2 M Ca(OAc) ₂
5	40% (v/v) PEG-300	Tris pH 7.0	5% (w/v) PEG-1000
6	30% (v/v) PEG-600	cacodylate pH 6.5	1 M NaCl, 10% (v/v) glycerol
7	40% (v/v) ethanol	Tris pH 7.0	none
8	35% (v/v) 2-ethoxyethanol	Na/K phosphate pH 6.2	0.2 M NaCl
9	35% (v/v) 2-propanol	imidazole pH 8.0	0.05 M Zn(OAc) ₂
10	40% (v/v) 1,2-propanediol	acetate pH 4.5	none
11	25% (v/v) 1, 2-propanediol	Na/K phosphate pH 6.2	10% (v/v) glycerol
12	40% (v/v) 1,2-propanediol	citrate pH 5.5	0.2 M NaCl
13	35% (v/v) 2-methyl-2,4-pentanediol	cacodylate pH 6.5	0.05 M Zn(OAc) ₂
14	40% (v/v) ethylene glycol	imidazole pH 8.0	0.2 M Ca(OAc) ₂
15	50% (v/v) PEG-200	Na/K phosphate pH 6.2	0.2 M NaCl
16	20% (v/v) PEG-300	imidazole pH 8.0	1 M (NH ₄) ₂ SO ₄ ,

Solution No.	precipitant	buffer (0.1 M)	additive(s)
			10% (v/v) glycerol
17	50% (v/v) PEG-400	MES pH 6.0	none
18	40% (v/v) PEG-300	phosphate-citrate pH 4.2	none
19	40% (v/v) PEG-600	acetate pH 4.5	0.2 M MgCl ₂
20	50% (v/v) ethylene glycol	CHES pH 9.5	0.5 M K/Na tartrate
21	35% (v/v) 2-ethoxyethanol	Tris pH 8.5	0.2 M Li ₂ SO ₄
22	35% (v/v) 2-propanol	cacodylate pH 6.5	0.2 M MgCl ₂
23	30% (v/v) 1,2-propanediol	HEPES pH 7.5	20% (v/v) PEG-400
24	25% (v/v) 1, 2-propanediol	Tris pH 8.5	0.2 M MgCl ₂ , 10% (v/v) glycerol
25	40% (v/v) 2-methyl-2,4-pentanediol	CAPS pH 10.5	none
26	40% (v/v) ethylene glycol	MES pH 6.0	0.2 M Zn(OAc) ₂
27	50% (v/v) PEG-200	Tris pH 7.0	none
28	40% (v/v) PEG-300	imidazole pH 8.0	0.2 M Zn(OAc) ₂
29	30% (v/v) PEG-400	HEPES pH 7.5	5% (w/v) PEG-3000, 10% (v/v) glycerol
30	40% (v/v) PEG-600	citrate pH 5.5	none
31	40% (v/v) PEG-600	CHES pH 9.5	none
32	35% (v/v) 2-propanol	acetate pH 4.5	none
33	45% (v/v) glycerol	cacodylate pH 6.5	0.2 M Ca(OAc) ₂
34	25% (v/v) 1, 2-propanediol	Tris pH 7.0	0.2 M (NH ₄) ₂ SO ₄ , 10% (v/v) glycerol
35	40% (v/v) 2-methyl-2,4-pentanediol	citrate pH 5.5	none
36	50% (v/v) PEG-200	cacodylate pH 6.5	0.2 M MgCl ₂
37	50% (v/v) ethylene glycol	imidazole pH 8.0	none

Solution No.	precipitant	buffer (0.1 M)	additive(s)
38	40% (v/v) PEG-400	acetate pH 4.5	none
39	30% (v/v) PEG-600	Tris pH 7.0	0.5 M $(\text{NH}_4)_2\text{SO}_4$, 10% (v/v) glycerol
40	40% (v/v) 2-methyl-2,4-pentanediol	CHES pH 9.5	none
41	50% (v/v) ethylene glycol	HEPES pH 7.5	0.2 M Li_2SO_4
42	30% (v/v) PEG-200	acetate pH 4.5	0.1 M NaCl
43	40% (v/v) PEG-400	imidazole pH 8.0	none
44	35% (v/v) 2-methyl-2,4-pentanediol	acetate pH 4.5	10% (v/v) glycerol
45	40% (v/v) PEG-300	acetate pH 4.5	0.2 M NaCl
46	30% (v/v) PEG-200	CAPS pH 10.5	0.2 M $(\text{NH}_4)_2\text{SO}_4$
47	50% (v/v) PEG-200	HEPES pH 7.5	none
48	50% (v/v) PEG-200	phosphate-citrate pH 4.2	0.2 M NaCl

Table III
Compositions of the Crystallization Solutions of Crystallization Solution Set III

Solution No.	precipitant	buffer (0.1 M)	salt (0.2 M)
1	20% (w/v) PEG-8000	CHES pH 9.5	none
2	10% (v/v) 2-propanol	HEPES pH 7.5	NaCl
3	15% (v/v) ethanol	CHES pH 9.5	none
4	35% (v/v) 2-methyl-2,4-pentanediol	imidazole pH 8.0	MgCl_2
5	30% (v/v) PEG-400	CAPS pH 10.5	none
6	20% (w/v) PEG-3000	citrate pH 5.5	none
7	10% (w/v) PEG-8000	MES pH 6.0	$\text{Zn}(\text{OAc})_2$
8	2.0 M $(\text{NH}_4)_2\text{SO}_4$	citrate pH 5.5	none
9	1.0 M $(\text{NH}_4)_2\text{HPO}_4$	acetate pH 4.5	none

Solution No.	precipitant	buffer (0.1 M)	salt (0.2 M)
10	20% (w/v) PEG-2000 MME	Tris pH 7.0	none
11	20% (v/v) 1,4-butanediol	MES pH 6.0	Li ₂ SO ₄
12	20% (w/v) PEG-1000	imidazole pH 8.0	Ca(OAc) ₂
13	1.26 M (NH ₄) ₂ SO ₄	cacodylate pH 6.5	none
14	1.0 M sodium citrate	cacodylate pH 6.5	none
15	10% (w/v) PEG-3000	imidazole pH 8.0	Li ₂ SO ₄
16	2.5 M NaCl	Na/K phosphate pH 6.2	none
17	30% (w/v) PEG-8000	acetate pH 4.5	Li ₂ SO ₄
18	1.0 M K/Na tartrate	imidazole pH 8.0	NaCl
19	20% (w/v) PEG-1000	Tris pH 7.0	none
20	0.4 M NaH ₂ PO ₄ /1.6 M K ₂ HPO ₄	imidazole pH 8.0	NaCl
21	20% (w/v) PEG-8000	HEPES pH 7.5	none
22	10% (v/v) 2-propanol	Tris pH 8.5	none
23	15% (v/v) ethanol	imidazole pH 8.0	MgCl ₂
24	35% (v/v) 2-methyl-2,4-pentanediol	Tris pH 7.0	NaCl
25	30% (v/v) PEG-400	Tris pH 8.5	MgCl ₂
26	10% (w/v) PEG-3000	CHES pH 9.5	none
27	1.2 M NaH ₂ PO ₄ /0.8 M K ₂ HPO ₄	CAPS pH 10.5	Li ₂ SO ₄
28	20% (w/v) PEG-3000	HEPES pH 7.5	NaCl
29	10% (w/v) PEG-8000	CHES pH 9.5	NaCl
30	1.26 M (NH ₄) ₂ SO ₄	acetate pH 4.5	NaCl
31	20% (w/v) PEG-8000	phosphate-citrate pH 4.2	NaCl
32	10% (w/v) PEG-3000	Na/K phosphate pH 6.2	none
33	2.0 M (NH ₄) ₂ SO ₄	CAPS pH 10.5	Li ₂ SO ₄
34	1.0 M (NH ₄) ₂ HPO ₄	imidazole pH 8.0	none
35	20% (v/v) 1,4-butanediol	acetate pH 4.5	none
36	1.0 M sodium citrate	imidazole pH 8.0	none

Solution No.	precipitant	buffer (0.1 M)	salt (0.2 M)
37	2.5 M NaCl	imidazole pH 8.0	none
38	1.0 M K/Na tartrate	CHES pH 9.5	Li ₂ SO ₄
39	20% (w/v) PEG-1000	phosphate-citrate pH 4.2	Li ₂ SO ₄
40	10% (v/v) 2-propanol	MES pH 6.0	Ca(OAc) ₂
41	30% (w/v) PEG-3000	CHES pH 9.5	none
42	15% (v/v) ethanol	Tris pH 7.0	none
43	35% (v/v) 2-methyl-2,4-pentanediol	Na/K phosphate pH 6.2	none
44	30% (v/v) PEG-400	acetate pH 4.5	Ca(OAc) ₂
45	20% (w/v) PEG-3000	acetate pH 4.5	none
46	10% (w/v) PEG-8000	imidazole pH 8.0	Ca(OAc) ₂
47	1.26 M (NH ₄) ₂ SO ₄	Tris pH 8.5	Li ₂ SO ₄
48	20% (w/v) PEG-1000	acetate pH 4.5	Zn(OAc) ₂

Table IV
Compositions of the Crystallization Solutions of Crystallization Solution Set IV

Solution No.	precipitant	buffer (0.1 M)	salt (0.2 M)
1	10% (w/v) PEG-3000	acetate pH 4.5	Zn(OAc) ₂
2	35% (v/v) 2-methyl-2,4-pentanediol	MES pH 6.0	Li ₂ SO ₄
3	20% (w/v) PEG-8000	Tris pH 8.5	MgCl ₂
4	2.0 M (NH ₄) ₂ SO ₄	cacodylate pH 6.5	NaCl
5	20% (v/v) 1,4-butanediol	HEPES pH 7.5	NaCl
6	10% (v/v) 2-propanol	phosphate-citrate pH 4.2	Li ₂ SO ₄
7	30% (w/v) PEG-3000	Tris pH 7.0	NaCl
8	10% (w/v) PEG-8000	Na/K phosphate pH 6.2	NaCl
9	2.0 M (NH ₄) ₂ SO ₄	phosphate-citrate pH 4.2	none
10	1.0 M (NH ₄) ₂ HPO ₄	Tris pH 8.5	none

Solution No.	precipitant	buffer (0.1 M)	salt (0.2 M)
11	10% (v/v) 2-propanol	cacodylate pH 6.5	Zn(OAc) ₂
12	30% (v/v) PEG-400	cacodylate pH 6.5	Li ₂ SO ₄
13	15% (v/v) ethanol	citrate pH 5.5	Li ₂ SO ₄
14	20% (w/v) PEG-1000	Na/K phosphate pH 6.2	NaCl
15	1.26 M (NH ₄) ₂ SO ₄	HEPES pH 7.5	none
16	1.0 M sodium citrate	CHES pH 9.5	none
17	2.5 M NaCl	Tris pH 7.0	MgCl ₂
18	20% (w/v) PEG-3000	Tris pH 7.0	Ca(OAc) ₂
19	1.6 M NaH ₂ PO ₄ /0.4 M K ₂ HPO ₄	phosphate-citrate pH 4.2	none
20	15% (v/v) ethanol	MES pH 6.0	Zn(OAc) ₂
21	35% (v/v) 2-methyl-2,4-pentanediol	acetate pH 4.5	none
22	10% (v/v) 2-propanol	imidazole pH 8.0	none
23	15% (v/v) ethanol	HEPES pH 7.5	MgCl ₂
24	30% (w/v) PEG-8000	imidazole pH 8.0	NaCl
25	35% (v/v) 2-methyl-2,4-pentanediol	HEPES pH 7.5	NaCl
26	30% (v/v) PEG-400	CHES pH 9.5	none
27	10% (w/v) PEG-3000	cacodylate pH 6.5	MgCl ₂
28	20% (w/v) PEG-8000	MES pH 6.0	Ca(OAc) ₂
29	1.26 M (NH ₄) ₂ SO ₄	CHES pH 9.5	NaCl
30	20% (v/v) 1,4-butanediol	imidazole pH 8.0	Zn(OAc) ₂
31	1.0 M sodium citrate	Tris pH 7.0	NaCl
32	20% (w/v) PEG-1000	Tris pH 8.5	none
33	1.0 M (NH ₄) ₂ HPO ₄	citrate pH 5.5	NaCl
34	10% (w/v) PEG-8000	imidazole pH 8.0	none
35	0.8 M NaH ₂ PO ₄ /1.2 M K ₂ HPO ₄	acetate pH 4.5	none
36	10% (w/v) PEG-3000	phosphate-citrate pH 4.2	NaCl
37	1.0 M K/Na tartrate	Tris pH 7.0	Li ₂ SO ₄

Solution No.	precipitant	buffer (0.1 M)	salt (0.2 M)
38	2.5 M NaCl	acetate pH 4.5	Li ₂ SO ₄
39	20% (w/v) PEG-8000	CAPS pH 10.5	NaCl
40	20% (w/v) PEG-3000	imidazole pH 8.0	Zn(OAc) ₂
41	2.0 M (NH ₄) ₂ SO ₄	Tris pH 7.0	Li ₂ SO ₄
42	30% (v/v) PEG-400	HEPES pH 7.5	NaCl
43	10% (w/v) PEG-8000	Tris pH 7.0	MgCl ₂
44	20% (w/v) PEG-1000	cacodylate pH 6.5	MgCl ₂
45	1.26 M (NH ₄) ₂ SO ₄	MES pH 6.0	none
46	1.0 M (NH ₄) ₂ HPO ₄	imidazole pH 8.0	NaCl
47	2.5 M NaCl	imidazole pH 8.0	Zn(OAc) ₂
48	1.0 M K/Na tartrate	MES pH 6.0	none

In another embodiment, the present invention includes subsets of the crystallization solutions of Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV. Presently preferred crystallization solution subsets are: subset I, solution numbers 1, 9, 10 and 28 of Crystallization Solution Set I; subset II, solution numbers 1, 8, 14, 26, 30 and 34 of Crystallization Solution Set II; subset III, solution numbers 2, 11, 18, 25, 26, 28, 29, 31, 38, 41 and 46 of Crystallization Solution Set III; and subset IV, solution numbers 2, 3, 5, 6, 7, 8, 17, 22, 24, 32, 34, 37, 43 and 48 of Crystallization Solution Set IV. Thus, crystallization solution subsets preferably include at least one of subset I, subset II, subset III and subset IV.

All of the crystallization solutions are made with ultrapure ASTM Type I water, and sterile-filtered into sterile tubes. The sterile crystallization solutions should be stored at room temperature. The following stock solutions are utilized to formulate the crystallization solutions.

Acetate pH 4.5. 1 M acetic acid and 1 M sodium acetate solutions are mixed together to make a pH 4.5 acetate stock solution. A 10-fold dilution of this stock solution is used in the final crystallization solution formulations, if required.

Cacodylate pH 6.5. A 1 M sodium cacodylate stock solution is adjusted to pH 6.5 with concentrated (37%) HCl. A 10-fold dilution of this stock solution is used in the final crystallization solution formulations, if required.

5 CAPS pH 10.5. A 1 M CAPS stock solution is adjusted to pH 10.5 with 50% (w/v) NaOH. A 10-fold dilution of this solution is used in the final crystallization solution formulations, if required.

CHES pH 9.5. A 1 M CHES stock solution is adjusted to pH 9.5 with 50% NaOH. A 10-fold dilution of this stock solution is used in the final crystallization solution formulations, if required.

10 Citrate pH 5.5. 0.5 M citric acid and 0.5 M sodium citrate solutions are mixed together to make a pH 5.5 citrate stock solution. A 5-fold dilution of this stock solution is used in the final crystallization solution formulations, if required.

15 HEPES pH 7.5. A 1 M HEPES stock solution is adjusted to pH 7.5 with 50% NaOH. A 10-fold dilution of this stock solution is used in the final crystallization solution formulations, if required.

Imidazole pH 8.0. A 1 M imidazole stock solution is adjusted to pH 8.0 with concentrated HCl. A 10-fold dilution of this stock solution is used in the final crystallization solution formulations, if required.

20 MES pH 6.0. A 1 M MES stock solution is adjusted to pH 6.0 with concentrated HCl. A 10-fold dilution of this stock solution is used in the final crystallization solution formulations, if required.

Na/K phosphate pH 6.2. 0.5 M Na_2HPO_4 and 0.5 M KH_2PO_4 solutions are mixed together to make a pH 6.2 Na/K phosphate stock solution. A 5-fold dilution of this stock solution is used in the final crystallization solution formulations, if required.

25 Phosphate-citrate pH 4.2. 0.5 M Na_2HPO_4 and 0.5 M citric acid solutions are mixed together to make a pH 4.2 phosphate-citrate stock solution. A 5-fold dilution of this stock solution is used in the final crystallization solution formulations, if required.

30 Tris pH 7.0 (or pH 8.5). A 1 M Tris base stock solution is adjusted to pH 7.0 or 8.5 with concentrated HCl. 10-fold dilutions of these stock solutions are used in the final crystallization solution formulations, if required.

35 When using the crystallization solutions of the present invention to crystallize a molecule, the molecule should be as highly purified as possible. If the molecule to be crystallized is a protein, preferably the protein should appear greater than 97% pure as determined by silver-stained SDS-PAGE. When the molecular sample is a

biological macromolecule, such as a protein, the molecular sample should be in as minimal a buffer as possible (i.e., the buffer should contain as few chemical components as possible) to help maintain the biological activity of the macromolecule, and the molecular sample should preferably be at a concentration of 5-15 mg/ml.

5 The crystallization solutions of the present invention can be used in any crystallization technique. Presently preferred crystallization techniques for use with the crystallization solutions of the present invention are vapor diffusion techniques as described, for example, in Gilliland, G.L. & Davies, D.R. (1984) *Methods in Enzymol.* 104:370-381; McPherson, A. (1990) *Eur. J. Biochem.* 189:1-23 and Weber, P.C.
10 (1991) *Adv. in Prot. Chem.* 41:1-36. For example, hanging drop crystallization is a vapor diffusion technique that typically utilizes crystallization plates including a plurality of reservoirs, such as those available from Hampton Research (27632 El Lazo Rd., Laguna Niguel, CA 92677) and ICN-Flow (3300 Hyland Ave., Costa Mesa, CA 92626). In an exemplary hanging drop crystallization experiment, sealant,
15 such as petroleum jelly or vacuum grease, is applied to the rim of a crystallization plate reservoir and 0.5-1.0 ml of a single crystallization solution of the present invention is pipetted into the reservoir. 1-10 µl (depending on availability) of the macromolecule sample is pipetted onto a siliconized cover slip (plates from Hampton Research and ICN-Flow typically require 22 mm square or round cover slips) and an
20 equal volume of the crystallization solution that is in the reservoir is added to the sample drop on the cover slip and mixed by repeatedly aspirating and dispensing the solution from the pipettor. The cover slip is inverted and sealed over the reservoir. When a crystallization solution set of the present invention is utilized, this sequence of events can be repeated for all 48 crystallization solutions in the crystallization solution
25 set.

Similarly, sitting drop crystallization is a type of vapor diffusion technique that utilizes sitting drop crystallization plates, including a plurality of reservoirs within each of which is located a pedestal that includes a sample depression within its upper end, such as those available from Charles Supper Co. (15 Tech Circle, Natick, MA 01760). In an exemplary sitting drop crystallization experiment utilizing the crystallization solutions of the present invention, 0.5-1.0 ml of a single crystallization solution are pipetted into a reservoir of a sitting drop crystallization plate and 1-10 µl of the sample are pipetted into the sample depression of the sitting drop pedestal. An equal volume of the crystallization solution that is in the reservoir is added to the sample drop and mixed. This procedure can be repeated, utilizing a different
30
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crystallization solution in each of the reservoirs. The reservoirs can be individually sealed with sealant and cover slips, or the entire sitting drop plate can be sealed with a single piece of clear sealing tape after application of sample to all wells has been completed.

5 By way of non-limiting example, other crystallization techniques that can utilize the crystallization solutions of the present invention include sandwich drop vapor diffusion which is similar to hanging drop and sitting drop vapor diffusion, except that the crystallization drop is contacted on two sides by glass or plastic surfaces. See, e.g., A. McPherson, *Eur. J. Biochem.* 189:1-23 (1990). Sandwich
10 drop crystallization plates are available from Hampton Research and ICN-Flow. In the technique of crystallization using oils, the rate of equilibration by vapor diffusion can be modulated by placing a layer of oil between the crystallization drop and the reservoir (see, e.g., Chayen, N.E. (1997) *J. Appl. Cryst.* 30:198-202). Alternatively, oils can be used to seal microbatch crystallization drops, in the absence of a larger
15 reservoir of crystallization solution (see, e.g., Chayen N.E. et al. (1990) *J. Appl. Cryst.* 23:297-302). In the technique of capillary crystallization, layers of sample solution and crystallization solution can be deposited in a capillary 0.5-1.0 mm in diameter, either with an air space between the solutions or with a direct liquid-liquid interface. Crystallization occurs by vapor diffusion or liquid-liquid diffusion inside the
20 capillary.

If the supply of sample permits, it is preferable to set up the crystallizations in duplicate, with one set of crystallizations placed at room temperature (typically from about 16°C to about 25°C), and the other one at 4°C. Regardless of the crystallization method used, the crystallization trials should preferably be stored in a place free of
25 vibrations or mechanical shock, which could result in premature precipitation.

Typically, observations of crystallization trials are recorded every one or two days. The crystallization trials can be viewed under a stereo microscope at 10-100x magnification. If less than ten percent of the samples in the crystallization screen do not show heavy precipitate after one day, it may be desirable to increase the
30 concentration of the sample molecule. If more than fifty percent of the samples in the crystallization screen show heavy precipitate after one day, it may be desirable to reduce the sample molecule concentration.

Crystals suitable for X-ray data collection are generally 0.1 mm or greater in their smallest dimension, and have clean, sharp edges. Viewing the crystallization trials between crossed polarizers often aids in distinguishing microcrystals from
35

amorphous precipitate. Except for the rather unusual occurrence of a cubic space group, X-ray diffraction quality biological macromolecule crystals are birefringent (have more than one refractive index), and turn polarized light. When rotated between crossed polarizers, the intensity and/or color of light transmitted through 5 birefringent crystals will change, with a periodicity of 90°. Amorphous precipitates will not transmit and turn polarized light.

If small crystals, or crystals which do not grow robustly in all three dimensions, are obtained in an initial screen, then a variety of art-recognized techniques can be utilized to obtain better crystals without undue experimentation. 10 For example, the crystallization conditions may be optimized by adjusting parameters, such as pH and temperature. Small crystals can be grown larger by seeding techniques (see, e.g., Thaller, C. et al. (1985) *Methods in Enzymol.* 114:132-135; Stura, E.A. & Wilson, I.A. (1990) *METHODS: A Companion to Methods in Enzymol.* 1:38-49). Using larger volume crystallization drops may also increase 15 crystal size (see, e.g., Fox, K.M. & Karplus, P.A. (1993) *J. Mol. Biol.* 234:502-507).

Ideally, crystals should be harvested straight from the crystallization drop and mounted for data collection. The solutions of Crystallization Solution Set I and Crystallization Solution Set II have been specially formulated so that they will freeze as a clear amorphous glass, and not interfere with the diffraction of macromolecule 20 crystals grown in these solutions. Thus, crystals may be mounted directly from the crystallization drop, and frozen in liquid nitrogen or a stream of gaseous nitrogen for cryo-temperature X-ray diffraction data collection (as described, for example, in Rodgers, D.W. (1994) *Structure*, 2:1135-1140). Again, crystallization conditions can be optimized, if necessary, by a variety of art-recognized techniques to generate very 25 high quality crystals. For example, cryo-stabilizers, such as glycerol, glucose, or PEG-400 can be added to the crystallization solutions of the present invention. Also, some macromolecule crystals may be damaged upon freezing, resulting in poor diffraction compared with their diffraction at room temperature. For such crystals, cross-linking reagents such as glutaraldehyde may be used to make the crystals more 30 resistant to mechanical stress upon freezing.

The four crystallization solution sets of the present invention have been shown to reproducibly crystallize proteins which are known to crystallize or are known to have a propensity to crystallize. These model proteins include apoferritin, thaumatin, lysozyme and canavalin. These readily crystallizable proteins represent a broad 35 spectrum of biochemical activities. For example, apoferritin is a large iron carrier

protein in blood, thaumatin is an especially sweet protein from potato, and lysozyme is from hen egg whites and is important for breaking down the glycoprotein shell of bacteria.

- Thus, for example, lysozyme crystallizes in solution number 28 of Crystallization Solution Set I; solution number 30 of Crystallization Solution Set II; solution numbers 28, 29, 31, 38 and 41 of Crystallization Solution Set III and in solution numbers 3, 7, 17, 24, 37, 43 and 48 of Crystallization Solution Set IV. Canavalin crystallizes in solution number 10 of Crystallization Solution Set I; solution numbers 2, 26 and 46 of Crystallization Solution Set III and in solution numbers 5, 8, 10 22 and 34 of Crystallization Solution Set IV. Thaumatin crystallizes in solution number 14 of Crystallization Solution Set II; solution numbers 18 and 38 of Crystallization Solution Set III and in solution number 37 of Crystallization Solution Set IV. Apoferritin crystallizes in solution numbers 1 and 26 of Crystallization Solution Set II.
- 15 The crystallization solutions of the present invention have also been used to successfully crystallize human topoisomerase I, a protein which is notoriously difficult to crystallize. Co-crystallization of human topoisomerase I, together with a 22 bp duplex DNA molecule, was achieved using solution number 25 of Crystallization Solution Set III, and permitted the structural determination of human 20 topoisomerase I. Subsequent work with a different topoisomerase I construct led to the identification of solution number 32 of Crystallization Solution Set IV as a good crystallization condition as well.

In another aspect, the present invention provides kits including a plurality of crystallization solutions of the present invention and at least one crystallization plate 25 that includes a plurality of reservoirs. Preferably the crystallization solutions are disposed within the reservoirs of the crystallization plates which can therefore be immediately used to conduct crystallization experiments. Any crystallization plates can be included in the kits of the present invention, including, by way of non-limiting example: Hampton Research plate models VDX, Linbro, Costar, Cryschem, Q-Plate, 30 Q-Plate II and Crystal Clear Strips; Charles Supper Co. sitting drop plates and ICN Linbro model. The presently preferred crystallization plates are disclosed in copending US Patent Application Serial Number 09/150,629, incorporated herein by reference.

By way of representative example, a presently preferred crystallization plate 35 for inclusion in a kit of the present invention is shown in FIGURES 1 and 2. With

reference to FIGURES 1 and 2, crystallization tray 10 includes a body 12 having an upper surface 14, a lower surface 16, a first end 18, a second end 20, a first side 22 and a second side 24. Body 12 defines a plurality of crystallization units 26. Each crystallization unit 26 includes a central reservoir 28, four diffusion channels 30 and 5 four drop chambers 32. Each drop chamber 32 is connected to central reservoir 28 by one diffusion channel 30. While the plate of FIGURES 1 and 2 is preferred, other plates or arrays of vessels may suitably be employed with the solutions of the present invention.

In a presently preferred embodiment, the present invention provides kits 10 including at least one crystallization plate and a set of crystallization solutions selected from the group of crystallization solution sets consisting of Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV. In another presently preferred embodiment, the present invention provides kits including at least one crystallization plate and a subset 15 of crystallization solutions selected from the group of subsets consisting of: subset I, solution numbers 1, 9, 10 and 28 of Crystallization Solution Set I; subset II, solution numbers 1, 8, 14, 26, 30 and 34 of Crystallization Solution Set II; subset III, solution numbers 2, 11, 18, 25, 26, 28, 29, 31, 38, 41 and 46 of Crystallization Solution Set III; and subset IV, solution numbers 2, 3, 5, 6, 7, 8, 17, 22, 24, 32, 34, 37, 43 and 20 48 of Crystallization Solution Set IV.

A kit of the present invention may optionally include, for example, water-permeable silicone oil DC200 (BDH, Gallard Schlesinger Industries, 584 Mineola Ave., Carle Place, NY 11514-1744, Catalogue number #63002 4N), and/or paraffin oil (Fluka Chemical Corp., 980 South 2nd St., Ronkonkoma, NY 11779-7238, catalogue number #76235) which are useful in microbatch crystallizations, and vapor diffusion crystallizations with oils.

The following examples merely illustrate the best mode now contemplated for practicing the invention, but should not be construed to limit the invention.

Example 1

Crystallization of Canavalin

Purified Canavalin (from Jack Bean) was kindly provided by Dr. Alex McPherson (University of California, Riverside). Protein slurry was dissolved by the addition of 1 M ammonium hydroxide until the solution appeared transparent. The sample was then adjusted to 200 mM sodium chloride by the addition of 5 M sodium chloride stock solution. The pH of the sample was then adjusted to pH 7.0 with the 30 35

addition of 0.1 M hydrochloric acid. The final concentration of protein was determined to be 30 milligrams per milliliter. This final protein solution was used in crystallization trials with Crystallization Solution Sets I, II, III and IV. The apparatus used for the experiments was the Compact Crystallization Plate (Emerald 5 Biostructures, 7869 N.E. Day Rd. W., Bainbridge Island, WA 98110).

The crystallization experiments were set up as follows. 0.2 milliliters of crystallization solution were placed into individual reservoir chambers of a Compact Crystallization Plate. Two microliters (μ l) of the crystallization solution were removed from the reservoir chambers and placed into individual drop chambers that 10 are connected to the reservoir chamber by a vapor diffusion channel. 2 μ l of the dissolved protein were then mixed with the 2 μ l of crystallisation solution in the drop chamber. The crystallization chambers were sealed with Crystal Clear tape (Manco, Inc., 32150 Just Imagine Drive, Avon OH 44011), and the plates were maintained at 14°C. Crystals formed within twelve to sixteen hours.

15 Canavalin crystallized in solution number 10 of Crystallization Solution Set I; solution numbers 2, 26 and 46 of Crystallization Solution Set III and in solution numbers 5, 8, 22 and 34 of Crystallization Solution Set IV.

Example 2

Crystallization of Lysozyme

20 Purified lysozyme (obtained from hen egg whites) was purchased from Sigma-Aldrich Co. (Catalogue number L7651). The protein powder was dissolved in water to a final concentration of 20 milligrams per milliliter (mg/ml). This final protein solution was used in crystallization trials with Crystallization Solution Sets I, II, III and IV exactly as described in Example 1 for Canavalin. Crystals formed within four 25 to five days. Lysozyme crystallized in solution number 28 of Crystallization Solution Set I; solution number 30 of Crystallization Solution Set II; solution numbers 28, 29, 31, 38 and 41 of Crystallization Solution Set III and in solution numbers 3, 7, 17, 24, 37, 43 and 48 of Crystallization Solution Set IV.

Example 3

Crystallization of Apoferritin

30 Purified Apoferritin (Horse Spleen) was purchased from Sigma-Aldrich Co. (Catalogue Number A4890). The protein solution was at 25 mg/ml and was used directly in crystallization trials with Crystallization Solution Sets I, II, III and IV exactly as described in Example 1 for Canavalin. Crystals formed within two to five

days. Apoferritin crystallized in solution numbers 1 and 26 of Crystallization Solution Set II.

Example 4

Crystallization of Thaumatin

- 5 Purified Thaumatin (purified from *Thaumatococcus daniellii*) was purchased from Sigma-Aldrich Co. (Catalogue number T7638). The protein powder was dissolved in water to a final concentration of 25 mg/ml. This final protein solution was used in crystallization trials with Crystallization Solution Sets I, II, III and IV exactly as described in Example 1 for Canavalin. Crystals formed within three to five days.
- 10 Thaumatin crystallized in solution number 14 of Crystallization Solution Set II; solution numbers 18 and 38 of Crystallization Solution Set III and in solution number 37 of Crystallization Solution Set IV.

Example 5

Crystallization of Topoisomerase I

- 15 Purified Human Topoisomerase I (70 kDa, amino acids 175 to 765 of the human topoisomerase I disclosed in P. D'Arpa et al., *Proc. Nat'l Acad. Sci. U.S.A.* **85**: 2543-2547 (1988), with an active site mutation of amino acid 723 from tyrosine to phenylalanine) was used in a crystallization trial with Crystallization Solution Sets I, II, III and IV as follows. The human Topoisomerase I was at 10 mg/ml in storage buffer (10 mM Tris-hydrochloric acid pH 7.5, 1 mM EDTA, 1 mM DTT). 0.6 milliliters of crystallization solution were placed into individual reservoir chambers of a Cryschem sitting drop crystallization plate (Charles Supper & Co., 15 Tech Circle, Natick, MA 01760). 2 µl of the topoisomerase I solution were placed into individual sitting drop chambers of the Cryschem plate. 1 µl of a 22 base-pair duplex oligonucleotide (0.2 mM duplex oligonucleotide in 6 mM sodium chloride) was then mixed with the 2 µl of Topoisomerase I. The sequence of the 5' to 3' strand of the duplex oligonucleotide is set forth in SEQ ID NO:1, and the sequence of the 3' to 5' strand of the duplex oligonucleotide is set forth in SEQ ID NO:2. 3µl of crystallization solution were then added to the topoisomerase-oligonucleotide mixture. The crystallization chambers were sealed with Crystal Clear tape, and the plates were maintained at 20°C. Crystals formed within three to five days.

The topoisomerase-oligonucleotide complex crystallized in solution number 25 of Crystallization Solution Set III, and in solution number 32 of Crystallization Solution Set IV.

While the preferred embodiment of the invention has been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A set of crystallization solutions comprising a set selected from the group of sets consisting of Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV.
2. A set of crystallization solutions of Claim 1 comprising Crystallization Solution Set I.
3. A set of crystallization solutions of Claim 2 consisting of Crystallization Solution Set I.
- 10 4. A set of crystallization solutions of Claim 1 comprising Crystallization Solution Set II.
5. A set of crystallization solutions of Claim 4 consisting of Crystallization Solution Set II.
6. A set of crystallization solutions of Claim 1 comprising Crystallization Solution Set III.
- 15 7. A set of crystallization solutions of Claim 6 consisting of Crystallization Solution Set III.
8. A set of crystallization solutions of Claim 1 comprising Crystallization Solution Set IV.
- 20 9. A set of crystallization solutions of Claim 8 consisting of Crystallization Solution Set IV.
10. A subset of crystallization solutions comprising a subset selected from the group of subsets consisting of solution numbers 1, 9, 10 and 28 of Crystallization Solution Set I, solution numbers 1, 8, 14, 26, 30 and 34 of Crystallization Solution Set II, solution numbers 2, 11, 18, 25, 26, 28, 29, 31, 38, 41 and 46 of Crystallization Solution Set III and solution numbers 2, 3, 5, 6, 7, 8, 17, 22, 24, 32, 34, 37, 43 and 25 48 of Crystallization Solution Set IV.

11. A subset of crystallization solutions of Claim 10 comprising solution numbers 1, 9, 10 and 28 of Crystallization Solution Set I.

12. A subset of crystallization solutions of Claim 10 comprising solution numbers 1, 8, 14, 26, 30 and 34 of Crystallization Solution Set II.

5 13. A subset of crystallization solutions of Claim 10 comprising solution numbers 2, 11, 18, 25, 26, 28, 29, 31, 38, 41 and 46 of Crystallization Solution Set III.

10 14. A subset of crystallization solutions of Claim 10 comprising solution numbers 2, 3, 5, 6, 7, 8, 17, 22, 24, 32, 34, 37, 43 and 48 of Crystallization Solution Set IV.

15 15. A kit comprising at least one crystallization plate and a set of crystallization solutions comprising a set selected from the group of sets consisting of Crystallization Solution Set I, Crystallization Solution Set II, Crystallization Solution Set III and Crystallization Solution Set IV.

16. The kit of Claim 15 wherein the set of crystallization solutions comprises Crystallization Solution Set I.

17. The kit of Claim 15 wherein the set of crystallization solutions comprises Crystallization Solution Set II.

18. The kit of Claim 15 wherein the set of crystallization solutions comprises Crystallization Solution Set III.

19. The kit of Claim 15 wherein the set of crystallization solutions comprises Crystallization Solution Set IV.

20. A kit comprising at least one crystallization plate and a subset of crystallization solutions comprising a subset selected from the group of subsets 25 consisting of solution numbers 1, 9, 10 and 28 of Crystallization Solution Set I, solution numbers 1, 8, 14, 26, 30 and 34 of Crystallization Solution Set II, solution numbers 2, 11, 18, 25, 26, 28, 29, 31, 38, 41 and 46 of Crystallization Solution Set III and solution numbers 2, 3, 5, 6, 7, 8, 17, 22, 24, 32, 34, 37, 43 and 48 of Crystallization Solution Set IV.

21. The kit of Claim 20 wherein the subset of crystallization solutions comprises solution numbers 1, 9, 10 and 28 of Crystallization Solution Set I.

22. The kit of Claim 20 wherein the subset of crystallization solutions comprises solution numbers 1, 8, 14, 26, 30 and 34 of Crystallization Solution Set II.

5 23. The kit of Claim 20 wherein the subset of crystallization solutions comprises solution numbers 2, 11, 18, 25, 26, 28, 29, 31, 38, 41 and 46 of Crystallization Solution Set III.

10 24. The kit of Claim 20 wherein the subset of crystallization solutions comprises solution numbers 2, 3, 5, 6, 7, 8, 17, 22, 24, 32, 34, 37, 43 and 48 of Crystallization Solution Set IV.

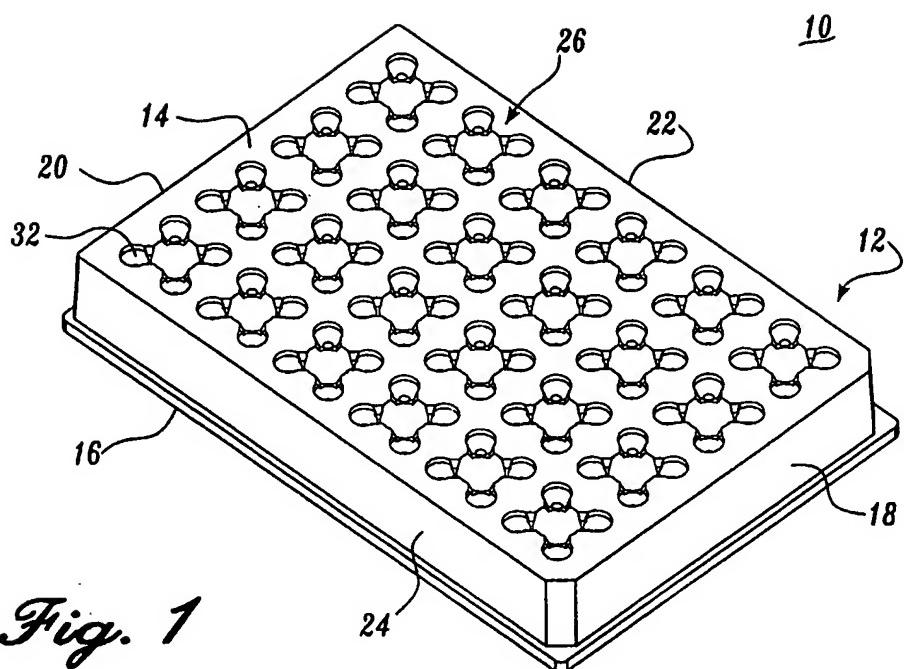


Fig. 1

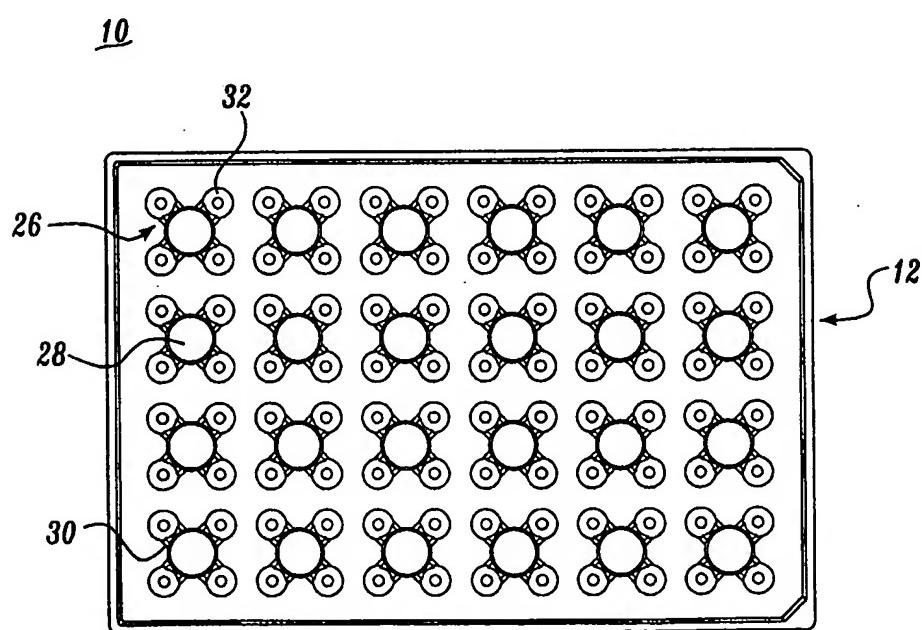


Fig. 2

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/14390

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C30B 35/00, 29/54

US CL :117/206, 925, 927; 422/61

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 117/206, 925, 927; 422/61

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

All electronic databases accessible via EAST (e.g., USPAT, Derwent, JPO, EPO)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,130,105A (Carter et al.) 14 July 1992	15-24
A	US 5,571,806A (Hargrave et al.) 05 November 1996	1-24
A	US 5,759,774A (Hackett et al.) 02 June 1998	1-24

 Further documents are listed in the continuation of Box C. See patent family annex.

Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A"	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

12 SEPTEMBER 1999

Date of mailing of the international search report

21 OCT 1999

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Education	The George Washington University Law School GPA 3.45 (With Honors) Juris Doctorate, May 1999
	University of Maryland at College Park GPA 3.85 (Magna Cum Laude) Bachelor of Science - Accounting, May 1995, Beta Alpha Psi (Honorary Accounting Fraternity)
Certifications	Bar Admissions: Maryland Bar (1999) & District of Columbia Bar (2000) Certified Public Accountant (1995)
Legal Experience	Securities & Exchange Commission, Office of Compliance Inspections and Examinations (Broker-Dealer Group), Senior Counsel, Washington DC - September 2003 to Present <ul style="list-style-type: none">• Lead and conduct examinations regarding broker-dealer issues, including market-timing/late trading, best execution, municipal bond/auction-rate securities and proprietary trading• Draft enforcement referrals, examination reports and planning memoranda• Initiate and create new examinations regarding hedge fund trading and municipal bonds• Draft memorandum to the Division of Market Regulation commenting on rule proposals• Conduct testimony of witnesses with the Division of Enforcement Dechert, Associate, Washington DC - September 1999 to September 2003 <ul style="list-style-type: none">• Advised clients and drafted memoranda and related compliance policies regarding Sarbanes-Oxley rules and regulations, including audit committee and certification requirements• Drafted registration statements and proxy materials for investment companies - primary contact for all registration statements for one of the largest bond fund investment advisers• Negotiated with SEC staff and drafted no-action request letter for clearing corporation seeking relief from the Investment Company Act of 1940• Prepared and drafted materials (resolutions, memoranda, minutes, etc.) for mutual fund board meetings• Prepared and drafted private placement memoranda and subscription documents for unregistered pooled investment vehicles Cadwalader, Wickersham & Taft, Summer Associate, Washington DC - Summer 1998 <ul style="list-style-type: none">• Conducted research and analysis of corporate, securities, bankruptcy, and environmental law• Prepared closing documents for a \$3 billion dollar securitization transaction• Drafted article related to environmental disclosure requirements in securitization transactions Federal Deposit Insurance Corporation - Office of Inspector General, Law Clerk, Washington DC - Summer 1997 <ul style="list-style-type: none">• Researched and analyzed criminal investigative practices and banking law• Drafted legal memoranda and letters to clients• Analyzed litigation documents to determine applicable privilege to assert
Business Experience	FundFusion, LLC, Founder and Chief Executive Officer - 2000 to 2001 <ul style="list-style-type: none">• Founded and developed an online investment management exchange• Drafted and presented business plan/concept to venture capitalists and angel investors Arthur Andersen LLP, Audit & Business Advisory Division, Staff Accountant/Auditor, Washington DC - July 1995 to August 1996 <ul style="list-style-type: none">• Prepared financial statements and related disclosures in conformity with GAAP• Evaluated overall control environment and performed tests of internal controls

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